

## Allergens are not pathogens

### Why immunization against allergy differs from vaccination against infectious diseases

Richard Weiss, Sandra Scheiblhofer, and Josef Thalhamer\*

<sup>1</sup>Department of Molecular Biology; University of Salzburg; Salzburg, Austria

Vaccination against infectious diseases has been one of the major breakthroughs in human medical history, saving the lives of millions of people each year. More recently, prophylactic vaccination against non-infectious diseases such as cancer, Alzheimer's disease, diabetes, and type I allergy is being investigated. Particularly in case of IgE-driven allergic disorders, which afflict almost a quarter of the population in highly developed countries, preventative measures would represent a major improvement for patients' health as well as an economic relief for public health services. As an alternative to allergen-specific immunotherapy, prophylactic vaccination against type I allergic diseases could slow down or even stop the progress of the allergy pandemic. Allergen-encoding gene-based vaccines, i.e., plasmid DNA and mRNA vaccines, provide the advantage of purity over crude allergen extracts, which involve the risk of *de novo* sensitizations. Furthermore, these formulations have been demonstrated to induce T helper 1 as well as T regulatory immune responses—a prerequisite for prophylactic intervention against allergies. However, prophylactic vaccines against environmental allergens strikingly differ from conventional vaccines against infectious diseases or therapeutic approaches concerning the underlying immunological mechanisms.

#### Prophylactic Vaccines Against Non-Infectious Diseases

In vaccinology the term “prophylactic” refers to a vaccine that will prevent a disease before its manifestation. While being common procedure for vaccines against pathogens, this concept has also been adopted for non-infectious diseases. In the case of cancer, prophylactic vaccines can be targeted at carcinogenic pathogens (e.g., HPV), tumor associated antigens (usually mutated proteins) as well as over-expressed self-antigens, which are no longer expressed in normal tissues (functional non-self proteins).<sup>1</sup> In this context, it has been suggested that such prophylactic vaccines against mutated-self could be applied to healthy individuals with either a genetic risk of cancer or bearing pre-neoplastic lesions.<sup>2</sup> Neurodegenerative diseases are another group of illnesses with a potential for prophylactic vaccine application. The disappointing outcome of clinical trials investigating therapeutic vaccination against amyloid  $\beta$  protein of Alzheimer's disease has been blamed on the late initiation of treatment and has prompted some investigators to suggest prophylactic vaccination of individuals at high risk<sup>3</sup> at an early age.<sup>4</sup> Similarly, prophylactic application of vaccines for prevention of type I diabetes has been considered, based on promising data from animal models.<sup>5</sup> However, it remains questionable whether such approaches would be ethically acceptable. In contrast to cancer or autoimmune diseases, vaccination against type I allergy does not involve administration of self-antigens. The advantages and potential

**Keywords:** DNA vaccine, mRNA vaccine, type I allergy, allergy vaccines, prophylactic, prevention, safety, natural immunity, infectious diseases, hygiene hypothesis

**Abbreviations:** Th1, T helper 1 cell; Th2, T helper 2 cell; Treg, T regulatory cell; Tr1, type 1 T regulatory cell; CTL, cytotoxic T cell

\*Correspondence to: Josef Thalhamer; Email: Josef.Thalhamer@sbg.ac.at

Submitted: 10/15/2013

Revised: 10/31/2013

Accepted: 11/13/2013

<http://dx.doi.org/10.4161/hv.27183>

pitfalls of prophylactic allergy vaccination are further discussed below.

### DNA and mRNA Vaccines

Gene vaccines have been celebrated as the next generation vaccines combining the safety of recombinant or subunit vaccines with the efficacy of live attenuated vaccines.<sup>6</sup> Like a live vector, gene vaccines are expressed *in vivo* and, as shown for self-replicating mRNA and DNA vaccines,<sup>7</sup> can even replicate in the host cell. Hence, not only antibody responses, but also CTL responses can be induced. While gene vaccines yielded promising results for a plethora of applications in animal models, and have already been licensed for veterinary use,<sup>8</sup> clinical trials in humans have been sobering so far.<sup>9</sup> However, while most early trials have aimed at therapeutic targets such as HIV or tumors, prophylactic vaccines against influenza have recently come into focus due to the H5N1 pandemic scare. Here, priming with DNA has been shown to expand and broaden the antibody repertoire against an inactivated vaccine and also to confer increased cross protection against heterologous strains.<sup>10,11</sup> These data substantiate previous findings that gene vaccines may be especially potent in priming a broad immune repertoire, which may later be recalled by a heterologous booster immunization.<sup>12</sup> This property makes DNA and mRNA vaccines promising candidates for the development of prophylactic vaccines against allergy.<sup>13</sup>

### Prophylactic Vaccines Against Allergies—Novel Ways to Battle a New Pandemic

Vaccination is one of the most efficient tools in human medicine with unsurpassed cost-benefits.<sup>14</sup> The steady increase of allergic diseases and the lack of adequate therapies pose a high burden not only on the quality of life of affected individuals, but also on public health services.<sup>15,16</sup> It is therefore reasonable to also apply prophylactic vaccination approaches to non-infectious pandemic diseases such as type I allergies. Immunization against allergy is usually associated with therapeutic intervention in already sensitized individuals.

This applies for classical specific immunotherapy with allergen extracts or, more recently with recombinant allergens, administered via subcutaneous injection or via the sublingual route using tablets or droplets.<sup>17</sup> The term “allergy vaccination” is frequently used to denote the application of specific immunotherapy (“allergy shots”), contributing to confusion of these 2 settings, which fundamentally differ with regards to the immunological situation.

Development of vaccines for the prevention of type I allergic diseases requires identification of the main allergen components. This can be achieved by population-wide testing for IgE reactivities against a multitude of allergen molecules using chip technology. It is known from population studies that depending on the geographical region, different allergens act as primary sensitizers,<sup>18</sup> which should be the key targets for protective immunizations. By preventing sensitization against these principal allergens, subsequent sensitizations can also be avoided. Therefore, careful selection of a few important sensitizers, adapted to regional needs, might be sufficient to provide broad protection.

Furthermore, instead of allergens in native conformation, as present in natural extracts, modified allergen derivatives with reduced potential to induce IgE responses have to be used in a prophylactic setting. As recently reviewed by Valenta et al., these include allergen-derived T-cell epitope containing peptides and recombinant hypoallergenic derivatives for induction of T-cell tolerance as well as carrier-bound B cell epitope-containing peptides to induce blocking IgG.<sup>19</sup> Given that allergic sensitization can be confined to a short period immediately after birth, prenatal or very early postnatal interventions have to be considered. Due to their exceptional safety profile, mRNA-based vaccines offer the possibility for immunomodulation early in life.<sup>20</sup>

### Different Requirements for Prevention of Infectious Diseases and Allergies

Most currently used vaccines against infectious diseases mediate the induction of high titer antibodies in serum or

at mucosal surfaces, which confer protection by blocking entry or limiting spread of bacteria or viruses. Vaccines targeting intracellular bacterial pathogens such as *Mycobacterium tuberculosis* additionally require cellular immune responses, i.e., the induction of CD4+ and CD8+ effector T cells. Furthermore, protection from short-incubation diseases correlates with effector memory functions, whereas for prevention of long-incubation diseases induction of central memory is essential.<sup>21</sup> Such potent immune responses are often only obtainable by the use of live-attenuated vaccines, or with powerful adjuvants.<sup>22</sup> Vaccination against infectious diseases therefore has always been a tightrope walk between inducing sterile protection and avoidance of intolerable side effects.

In contrast, prophylactic immunization against type I allergy does not require the induction of neutralizing antibody titers or powerful CD8+ responses. We and others have demonstrated that subtle priming of the immune system with allergen-encoding genetic vaccines suffices for protection against subsequent sensitization. Recurrent contact with the respective allergen(s) will preserve and stabilize the initial vaccine-driven response. Even very low doses of DNA<sup>23,24</sup> or mRNA<sup>25</sup> vaccines that prime barely detectable immune responses set an adequate immune bias, which is expanded by subsequent (natural) exposure to the allergen, e.g., seasonal aerosol exposure to pollen allergens. This closely resembles to lessons learned about the efficacy of vaccines against infectious diseases: the presence of the pathogen boosts vaccination responses and thus contributes to the maintenance of memory.<sup>26</sup>

### Protective Immunity Against Allergy

In contrast to acquired immunity against infectious diseases, which is mostly antibody dependent, protective immunity against allergies appears to be CD4+ T-cell mediated. While allergic individuals display a clearly Th2 biased immune polarization, responses by non-atopic, asymptomatic individuals are mainly of the Tr1 (IL-10 secreting)<sup>27,28</sup> or Th1/Tr1 (IFN- $\gamma$  and IL-10 secreting)<sup>29,30</sup>

type, indicating that true immunological ignorance might not exist. As initially proposed by the so-called hygiene hypothesis, Th1 immune deviation early in life provides a mechanism to suppress allergic sensitization against environmental allergens.<sup>31</sup> Although this hypothesis may be untenable in this narrowly defined version,<sup>32</sup> it is now well accepted that besides immunological tolerance, induction of Th1-biased immunity against allergens represents an additional strategy exploited by nature to induce normal healthy immunity instead of exaggerated IgE-driven allergic responses. We have recently found that of 71 non-atopic donors, who grew up in a farming environment, 14% mounted IFN- $\gamma$  responses against the grass pollen allergen Phl p 5, 10% IL-10, 25% mixed IFN- $\gamma$ /IL-10, 6% IL-22, 10% IL-6, and 3% IL-17 responses, while the remaining 32% displayed only IL-2 or none of the tested cytokines (unpublished observation).

Both, regulatory<sup>33-35</sup> as well as Th1<sup>23-25</sup> T-cell responses have been shown to be induced by genetic vaccines, protecting from allergic sensitization in animal models. Therefore, prophylactic vaccination against allergy can be regarded as a true protective vaccination mimicking one of the strategies nature uses to mount healthy immune responses. Moreover, recruitment of allergen-specific memory T cells provides protection without the necessity for native conformation of the encoded allergen molecules. To avoid unwanted exposure to B-cell epitopes and the potential induction of IgE antibodies, the sequence of the encoded allergen can be easily modified.<sup>36</sup> Alternatively, translation of a gene vaccine can be targeted either to the proteasome<sup>35,37</sup> or the endosome.<sup>35</sup> Notably, the latter strategy has been recently employed in the first phase I clinical study, which investigates the safety of an allergen-encoding DNA vaccine.<sup>38</sup>

### **Safety and Efficacy of Allergy Vaccines**

As prophylactic allergy gene vaccines require only subtle immune priming, safety aspects can be granted highest priority. This can be achieved by administration of low doses of plasmid DNA, which

would be otherwise regarded suboptimal. In case of self-replicating vaccines, even lower doses can be applied.<sup>23,25,39</sup> mRNA vaccines, which are generally regarded to provide lower immunogenicity, represent a promising alternative with expression limited to a short time-window.<sup>25</sup> In contrast to therapeutic vaccines or vaccines against infectious diseases, formulations for prophylactic allergy vaccines likely will not have to include adjuvants or require special delivery systems, which are frequently responsible for side-effects, posing a major problem for modern vaccinology.

Following minimal stimulation of the immune system by early intervention, boosting of the established immune response can be achieved by environmental exposure. In this context, exact timing of the primary immunization might be crucial to exploit re-stimulation by natural allergen exposure. Of course, this only applies for seasonal (pollen) allergens and not perennial allergens derived from dust mite or animal dander. By taking advantage of this natural prime-boost regimen, the primary vaccination may be limited to a single injection without the requirement for booster immunizations.

Following initial studies demonstrating the anti-allergic potential of gene vaccines, which clearly linked this capacity to the induction of Th1 immunity, apprehension was raised that this type of response might trigger pathological inflammation, particularly of the airways, and could even lead to development of autoimmunity. However, the fact that Th1 responses induced by DNA or mRNA immunization are at the very most of moderate strength, taken together with positive safety data from more than 140 clinical trials, have changed the perception and gene vaccines are no longer regarded to pose a risk for such side effects. This notion has been encouraged by studies, which could not detect exaggerated Th1 driven responses,<sup>23-25</sup> confirming the self-limiting capacity of Th1 cells by autologous IL-10 secretion.<sup>40,41</sup> Notably, IFN- $\gamma$ /IL-10 secreting CD4<sup>+</sup> T cells have not only been found in non-atopic individuals,<sup>29,30</sup> but also after successful completion of sublingual immunotherapy.<sup>42</sup> Moreover, we have recently demonstrated in a mouse model of allergic asthma that

the initially induced protective Th1-bias did not lead to the emergence of pathological Th1-mediated inflammation, even after repeated monthly exposure to aerosolized allergen (unpublished data).

### **Considerations for Trial Design**

Clinical evaluation of prophylactic vaccines against allergy implies vaccination of a large cohort of healthy subjects, which have to be followed for a prolonged period of time. With respect to vaccine design, an unbiased recruitment of study participants would not be feasible due to financial considerations. Therefore, the most important step would be to select a sizeable cohort of young children with high predictability of developing type I allergies. This can be achieved by utilizing family anamnesis, genetic markers,<sup>43</sup> and diagnosis of certain food allergies early in life<sup>44</sup> or of primary sensitizations preceding allergies to other respiratory allergens.<sup>45</sup>

### **Concluding Remarks**

DNA, and more recently, mRNA vaccines have come of age. Initial problems regarding lack of immunogenicity and safety concerns have been addressed in numerous clinical trials. Gene based vaccines are now ready for the next step toward application in non-life threatening diseases. The first DNA vaccine encoding an allergen has recently demonstrated its safety in a phase I clinical trial. However, while this vaccine is still designed for therapeutic application, we believe that the greatest strength of allergy gene vaccines, and especially mRNA vaccines, lies in their prophylactic application. The available preclinical data convincingly support the concept of mRNA based prophylactic allergy vaccination, and clinical evaluation is now the next logical step.

#### **Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

#### **Acknowledgments**

This work has been supported by the Austrian Science Fund (FWF, project #W1213), the Christian Doppler

## References

1. Tuohy VK, Jaini R. Prophylactic cancer vaccination by targeting functional non-self. *Ann Med* 2011; 43:356-65; PMID:21651440; <http://dx.doi.org/10.3109/07853890.2011.565065>
2. Finn OJ, Forni G. Prophylactic cancer vaccines. *Curr Opin Immunol* 2002; 14:172-7; PMID:11869888; [http://dx.doi.org/10.1016/S0952-7915\(02\)00317-5](http://dx.doi.org/10.1016/S0952-7915(02)00317-5)
3. McCarron MO, Nicoll JA. Cerebral amyloid angiopathy and thrombolysis-related intracerebral haemorrhage. *Lancet Neurol* 2004; 3:484-92; PMID:15261609; [http://dx.doi.org/10.1016/S1474-4422\(04\)00825-7](http://dx.doi.org/10.1016/S1474-4422(04)00825-7)
4. Shimada M, Abe S, Takahashi T, Shiozaki K, Okuda M, Mizukami H, Klinman DM, Ozawa K, Okuda K. Prophylaxis and treatment of Alzheimer's disease by delivery of an adeno-associated virus encoding a monoclonal antibody targeting the amyloid Beta protein. *PLoS One* 2013; 8:e57606; PMID:23555563; <http://dx.doi.org/10.1371/journal.pone.0057606>
5. Li A, Escher A. DNA Immunotherapies for Type 1 Diabetes. In: Escher A, ed. *Endocrinology and Metabolism: Type 1 Diabetes*, 2013.
6. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? *Nat Rev Genet* 2008; 9:776-88; PMID:18781156; <http://dx.doi.org/10.1038/nrg2432>
7. Geall AJ, Mandl CW, Ulmer JB. RNA: the new revolution in nucleic acid vaccines. *Semin Immunol* 2013; 25:152-9; PMID:23735226; <http://dx.doi.org/10.1016/j.smim.2013.05.001>
8. Redding L, Weiner DB. DNA vaccines in veterinary use. *Expert Rev Vaccines* 2009; 8:1251-76; PMID:19722897; <http://dx.doi.org/10.1586/erv.09.77>
9. Ferraro B, Morrow MP, Hutnick NA, Shin TH, Lucke CE, Weiner DB. Clinical applications of DNA vaccines: current progress. *Clin Infect Dis* 2011; 53:296-302; PMID:21765081; <http://dx.doi.org/10.1093/cid/cir334>
10. Ledgerwood JE, Wei CJ, Hu Z, Gordon IJ, Enama ME, Hendel CS, McTamney PM, Pearce MB, Yassine HM, Boyington JC, et al.; VRC 306 Study Team. DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. *Lancet Infect Dis* 2011; 11:916-24; PMID:21975270; [http://dx.doi.org/10.1016/S1473-3099\(11\)70240-7](http://dx.doi.org/10.1016/S1473-3099(11)70240-7)
11. Ledgerwood JE, Zephir K, Hu Z, Wei CJ, Chang L, Enama ME, Hendel CS, Sitar S, Bailor RT, Koup RA, et al.; VRC 310 Study Team. Prime-boost interval matters: a randomized phase 1 study to identify the minimum interval necessary to observe the H5 DNA influenza vaccine priming effect. *J Infect Dis* 2013; 208:418-22; PMID:23633407; <http://dx.doi.org/10.1093/infdis/jit180>
12. Lu S. Heterologous prime-boost vaccination. *Curr Opin Immunol* 2009; 21:346-51; PMID:19500964; <http://dx.doi.org/10.1016/j.coi.2009.05.016>
13. Weiss R, Scheibelhofer S, Roesler E, Ferreira F, Thalhamer J. Prophylactic mRNA vaccination against allergy. *Curr Opin Allergy Clin Immunol* 2010; 10:567-74; PMID:20856111; <http://dx.doi.org/10.1097/ACI.0b013e32833fd5b6>
14. Ozawa S, Mirelman A, Stack ML, Walker DG, Levine OS. Cost-effectiveness and economic benefits of vaccines in low- and middle-income countries: a systematic review. *Vaccine* 2012; 31:96-108; PMID:23142307; <http://dx.doi.org/10.1016/j.vaccine.2012.10.103>
15. Meltzer EO, Bukstein DA. The economic impact of allergic rhinitis and current guidelines for treatment. *Annals of allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology* 2011; 106:S12-6
16. Bahadori K, Doyle-Waters MM, Marra C, Lynd L, Alasaly K, Swiston J, FitzGerald JM. Economic burden of asthma: a systematic review. *BMC Pulm Med* 2009; 9:24; PMID:19454036; <http://dx.doi.org/10.1186/1471-2466-9-24>
17. Burks AW, Calderon MA, Casale T, Cox L, Demoly P,utel M, Nelson H, Akdis CA. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J Allergy Clin Immunol* 2013; 131:1288-96, e3; PMID:23498595; <http://dx.doi.org/10.1016/j.jaci.2013.01.049>
18. Fernández-Rivas M, Bolhaar S, González-Mancebo E, Asero R, van Leeuwen A, Bohle B, Ma Y, Ebner C, Rigby N, Sancho AI, et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006; 118:481-8; PMID:16890775; <http://dx.doi.org/10.1016/j.jaci.2006.05.012>
19. Valenta R, Campana R, Marth K, van Hage M. Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches. *J Intern Med* 2012; 272:144-57; PMID:22640224; <http://dx.doi.org/10.1111/j.1365-2796.2012.02556.x>
20. Weiss R, Scheibelhofer S, Roesler E, Weinberger E, Thalhamer J. mRNA vaccination as a safe approach for specific protection from type I allergy. *Expert Rev Vaccines* 2012; 11:55-67; PMID:22149709; <http://dx.doi.org/10.1586/erv.11.168>
21. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol* 2010; 17:1055-65; PMID:20463105; <http://dx.doi.org/10.1128/CVI.00131-10>
22. Titball RW. Vaccines against intracellular bacterial pathogens. *Drug Discov Today* 2008; 13:596-600; PMID:18598915; <http://dx.doi.org/10.1016/j.drudis.2008.04.010>
23. Gabler M, Scheibelhofer S, Kern K, Leitner WW, Stoecklinger A, Hauser-Kronberger C, Alinger B, Lechner B, Prinz M, Vrtala S, et al. Immunization with a low-dose replicon DNA vaccine encoding Phl p 5 effectively prevents allergic sensitization. *J Allergy Clin Immunol* 2006; 118:734-41; PMID:16950295; <http://dx.doi.org/10.1016/j.jaci.2006.04.048>
24. Pulsawat P, Pitakpolrat P, Prompetchara E, Kaewamatawong T, Techakriengkrai N, Sirivichayakul S, Buranapraditkun S, Hannaman D, Ruxrutham K, Jaquet A. Optimization of a Der p 2-based prophylactic DNA vaccine against house dust mite allergy. *Immunol Lett* 2013; 151:23-30; PMID:23396105; <http://dx.doi.org/10.1016/j.imlet.2013.01.013>
25. Roesler E, Weiss R, Weinberger EE, Fruehwirth A, Stoecklinger A, Mostböck S, Ferreira F, Thalhamer J, Scheibelhofer S. Immunize and disappear-safety-optimized mRNA vaccination with a panel of 29 allergens. *J Allergy Clin Immunol* 2009; 124:1070-7, e1-11; PMID:19665781; <http://dx.doi.org/10.1016/j.jaci.2009.06.036>
26. Zinkernagel RM. Immunological memory ≠ protective immunity. *Cell Mol Life Sci* 2012; 69:1635-40; PMID:22481438; <http://dx.doi.org/10.1007/s00018-012-0972-y>
27. Smith KA, Gray NJ, Cheek E, Saleh F, Lavender J, Frew AJ, Kern J, Tarzi MD. Characterisation of CD154+ T cells following ex vivo birch allergen stimulation defines a close relationship between T cell subsets in healthy volunteers. *BMC Immunol* 2013; 14:14; PMID:23521868; <http://dx.doi.org/10.1186/1471-2172-14-14>
28. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Cramer R, Thunberg S, Deniz G, Valenta R, Fiebig H, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; 199:1567-75; PMID:15173208; <http://dx.doi.org/10.1084/jem.20032058>
29. Van Overtvelt L, Wambre E, Maillère B, von Hofe E, Louise A, Balazuc AM, Bohle B, Ebo D, Leboulleire C, Garcia G, et al. Assessment of Bet v 1-specific CD4+ T cell responses in allergic and nonallergic individuals using MHC class II peptide tetramers. *J Immunol* 2008; 180:4514-22; PMID:18354173
30. Bullens DM, De Swert A, Dilissen E, Kasran A, Kroczeck RA, Cadot P, Casar P, Ceuppens JL. House dust mite-specific T cells in healthy non-atopic children. *Clin Exp Allergy* 2005; 35:1535-41; PMID:16393318; <http://dx.doi.org/10.1111/j.1365-2222.2005.02352.x>
31. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004; 112:352-63; PMID:15196202; <http://dx.doi.org/10.1111/j.1365-2567.2004.01925.x>
32. Brooks C, Pearce N, Douwes J. The hygiene hypothesis in allergy and asthma: an update. *Curr Opin Allergy Clin Immunol* 2013; 13:70-7; PMID:23103806; <http://dx.doi.org/10.1097/ACI.0b013e32835ad0d2>
33. Pei Y, Geng S, Liu L, Yan F, Guan H, Hou J, Chen Y, Wang B, An X. Fel d 1-airway inflammation prevention and treatment by co-immunization vaccine via induction of CD4+CD25-Foxp3+ Treg cells. *Hum Vaccin Immunother* 2013; 9: 1019-31; PMID:23324570; <http://dx.doi.org/10.4161/hv.23518>
34. Jin H, Kang Y, Zhao L, Xiao C, Hu Y, She R, Yu Y, Du X, Zhao G, Ng T, et al. Induction of adaptive T regulatory cells that suppress the allergic response by coimmunization of DNA and protein vaccines. *J Immunol* 2008; 180:5360-72; PMID:18390718
35. Weinberger EE, Isakovic A, Scheibelhofer S, Ramsauer C, Reiter K, Hauser-Kronberger C, Thalhamer J, Weiss R. The influence of antigen targeting to subcellular compartments on the anti-allergic potential of a DNA vaccine. *Vaccine* 2013; Forthcoming; PMID:23954383; <http://dx.doi.org/10.1016/j.vaccine.2013.08.005>
36. Thalhamer T, Dobias H, Stepanoska T, Pröll M, Stutz H, Dissertori O, Lackner P, Ferreira F, Wallner M, Thalhamer J, et al. Designing hypoallergenic derivatives for allergy treatment by means of in silico mutation and screening. *J Allergy Clin Immunol* 2010; 125:926-34, e10; PMID:20371399; <http://dx.doi.org/10.1016/j.jaci.2010.01.031>
37. Bauer R, Scheibelhofer S, Kern K, Gruber C, Stepanoska T, Thalhamer T, Hauser-Kronberger C, Alinger B, Zoegg T, Gabler M, et al. Generation of hypoallergenic DNA vaccines by forced ubiquitination: preventive and therapeutic effects in a mouse model of allergy. *J Allergy Clin Immunol* 2006; 118:269-76; PMID:16815165; <http://dx.doi.org/10.1016/j.jaci.2006.03.033>
38. NCT01707069. A Safety and Immunogenicity Phase I Study of CryJ2-DNA-Lysosomal Associated Membrane Protein (CryJ2 -DNA-LAMP) Plasmid. 2012; <http://clinicaltrials.gov/show/NCT01707069>.
39. Scheibelhofer S, Gabler M, Leitner WW, Bauer R, Zoegg T, Ferreira F, Thalhamer J, Weiss R. Inhibition of type I allergic responses with nanogram doses of replicon-based DNA vaccines. *Allergy* 2006; 61:828-35; PMID:16792580; <http://dx.doi.org/10.1111/j.1398-9995.2006.01142.x>

40. Jankovic D, Kullberg MC, Feng CG, Goldszmid RS, Collazo CM, Wilson M, Wynn TA, Kamanaka M, Flavell RA, Sher A. Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J Exp Med* 2007; 204:273-83; PMID:17283209; <http://dx.doi.org/10.1084/jem.20062175>
41. Gabrysová L, Nicolson KS, Streeter HB, Verhagen J, Sabatos-Peyton CA, Morgan DJ, Wraith DC. Negative feedback control of the autoimmune response through antigen-induced differentiation of IL-10-secreting Th1 cells. *J Exp Med* 2009; 206:1755-67; PMID:19635862; <http://dx.doi.org/10.1084/jem.20082118>
42. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007; 120:707-13; PMID:17681368; <http://dx.doi.org/10.1016/j.jaci.2007.06.013>
43. Marenholz I, Kerscher T, Bauerfeind A, Esparza-Gordillo J, Nickel R, Keil T, Lau S, Rohde K, Wahn U, Lee YA. An interaction between filaggrin mutations and early food sensitization improves the prediction of childhood asthma. *J Allergy Clin Immunol* 2009; 123:911-6; PMID:19348926; <http://dx.doi.org/10.1016/j.jaci.2009.01.051>
44. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999; 103:1173-9; PMID:10359902; [http://dx.doi.org/10.1016/S0091-6749\(99\)70195-8](http://dx.doi.org/10.1016/S0091-6749(99)70195-8)
45. Hatzler L, Panetta V, Lau S, Wagner P, Bergmann RL, Illi S, Bergmann KE, Keil T, Hofmaier S, Rohrbach A, et al. Molecular spreading and predictive value of preclinical IgE response to *Phleum pratense* in children with hay fever. *J Allergy Clin Immunol* 2012; 130:894-901, e5; PMID:22841010; <http://dx.doi.org/10.1016/j.jaci.2012.05.053>